Visualization of the Passive Sink Phenomenon in Nonexercising Lower Extremity Muscle Using Two Sampling Sites: Consequences for Assessment and Training

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DEDICATION

I dedicate this thesis to my father, James Michael Copolo. When people tell me I am just like you, it is the highest compliment I could ever receive. This dedication is the smallest compliment I could ever give you for the lifetime of love, support, and most importantly friendship.

I also dedicate this thesis to my mother, Karen Sue Copolo. You are the perfect example of beating the odds, and I am proud to be your one in a million. God may have given you a daughter, but he gave me a best friend and the strongest woman I know as a role model.
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ABSTRACT

In the past, interest in the application of human performance and testing values has been limited to exercise professionals and those who participate in physical activity at high levels. One common method exercise physiologists employ to determine performance is the assessment of blood lactate concentration [La'] through sampling. Blood lactate has been studied extensively; however, selection of an optimal sample site for drawing blood lactate is still an ongoing concern for exercise physiologists.

Site selection can impact the values of blood [La'] being reported, which can in turn impact the development of the training prescription. Studies that compare a proximal and distal sample site were few until (Comeau, Lawson, Graves, Church, & Adams, 2011) performed a study using common sampling practices to visualize the passive sink phenomenon in non-exercising upper extremity muscles after a bout of exhaustive lower extremity exercise. The purpose of this study is to determine if the passive sink phenomenon can be visualized in the lower extremity during upper extremity exercise. Seven college-aged males completed a 15·W·min⁻¹ incremental exercise protocol at 60 rpm to volitional fatigue on an upper body ergometer. Blood [La'] was measured from the finger and toe at rest immediately post-exercise and every five minutes thereafter for 30 minutes after the exercise bout. A two-way 2 x 7 (site x sample time) within- subjects repeated-measures ANOVA determined no significant interaction effect. A significant time main effect did exist with Wilks’ Lambda = .032 (F₆,₇ = 35.114, p=0.000). Blood lactate levels should be assessed from samples taken from the limb proximal to the exercising extremity to negate the passive sink effect when establishing training protocols from blood [La'].
Chapter 1

INTRODUCTION

In the past, interest in the application of human performance and testing values has been limited to exercise professionals and those who participate in physical activity at high levels. However, in a time when people are becoming more aware and educated to the ways physiological testing results can improve their athletic performance, everyone from the weekend warrior to the elite marathoner is interested in how to apply exercise testing to his or her training regimen. One common method exercise physiologists employ to determine performance is the assessment of blood lactate concentration [La\(^{-}\)] through sampling. Blood lactate has been studied extensively; however, selection of an optimal sample site for drawing blood lactate is still an ongoing concern for exercise physiologists.

Site selection can impact the values of blood [La\(^{-}\)] being reported, which can in turn impact the development of the training prescription. The mode of exercise, the duration and intensity of exercise can also impact reported blood [La\(^{-}\)]. One factor that can contribute to discrepancies of reported blood [La\(^{-}\)] is the role that inactive muscle plays on the metabolic fate of lactate uptake.

There have been numerous studies conducted in which the measurement of blood lactate concentration [La\(^{-}\)] has been performed through blood sampling taken from sites proximal to the muscle group performing work, or taken from sites distal to the muscle group performing work (Ahlborg, Hagenfeldt, & Wahren, 1975; Forsyth & Farrally, 2000; Garland & Atkinson, 2008; Karlsson, Bonde-Petersen, Henriksson, & Knuttgen, 1975; Poortmans, Delescaille-Vanden Bossche, & Leclercq, 1978). However, studies
that compare a proximal and distal sample site were few until (Comeau, et al., 2011) performed a study using common sampling practices to visualize the passive sink phenomenon in non-exercising upper extremity muscles after a bout of exhaustive lower extremity exercise. When a sample of blood taken from a site closer to the non-exercising muscle group has a higher [La−] than blood sampled from a site closer to the exercising muscle group, the phenomenon known as passive sink is visible (Comeau, et al., 2011). The presence of this phenomenon seeks to explain the accuracy of the correlation between the sampling site selection and muscle group being utilized during exercise. The passive sink phenomenon has never been visualized in the non-exercising lower extremity muscles when upper extremity muscles have performed a bout of exhaustive exercise.

STATEMENT OF THE PROBLEM

This study is designed to measure [La−] from a site close to and a site away from the exercising muscle utilizing common sampling methods in order to visualize the passive sink effect in the non-exercising lower extremity. The problem is to apply the concepts used by (Comeau, et al., 2011) to obtain blood from exercising and non-exercising limb, and analyze the lactate concentration, in order to determine the role that the sampling site plays in reported [La−] values.

PURPOSE OF THE STUDY

Blood lactate sampling has been utilized as a method for evaluating athletic performance and developing training prescriptions for many years (Moran, Prichard, Ansley, & Howatson, 2012). However, as our understanding of blood [La−] increases, the
question of sample site selection gains in importance, in order for the results to reflect the most accurate representation of blood [La'].

There have been many sites utilized as sample sites for drawing blood lactate. Other than the typical venous and arterial blood draws, ear lobe, finger and great toe sticks have been employed (Ahlborg, et al., 1975; Baker, Brown, Hill, Phillips, Williams, & Davies, 2002). There has been significant variability between sample sites as well as variability related to the time at which the sample is taken after an exercise bout. Given the importance of sample site selection and due to the key role the data play in determining a training prescription, it is crucial that we attain an accurate understanding of the role inactive muscle plays on [La']. The passive sink phenomenon occurs when lactate pools in the inactive muscle, which can lead to higher lactate levels in sites closer to the non-active muscle than in sites closer to the active muscle (Poortmans, et al., 1978). The presence of a passive sink phenomenon in the upper extremity during lower extremity exercise has been visualized by Comeau and colleagues (2011) in a previous work; therefore, the purpose of this study is to determine if the passive sink phenomenon can be visualized in the lower extremity during upper extremity exercise.

SIGNIFICANCE OF THE STUDY

As we investigate the relationship between sampling site and blood [La'] and attempt to relate it to the pattern of lactate appearance or disappearance, several factors come into play. The site from which the blood is drawn is of vital importance when the resultant data are being utilized to prescribe an exercise training protocol (Comeau, et al., 2011; Moran, et al., 2012). This study is significant in that it further reveals information on the metabolic activity of a resting muscle group before, during and after exhaustive
exercise with other muscle groups. There is a not a great deal of information about the metabolic processes of the inactive muscle during exercise (Ahlborg, et al., 1975; Poortmans, et al., 1978). Whereas blood lactate was once thought of as a metabolic “dead end” responsible for fatigue, it is now evident that lactate plays a much more useful role in intermediary metabolism (Brooks, 1986; Brooks & Gaesser, 1980; Gladden, 2004; Poortmans, et al., 1978). The fate of this metabolite is much debated, and tracer studies by Brooks were performed in order to determine the metabolic pathways of lactate transport after an exhaustive bout of exercise (Brooks & Gaesser, 1980). This groundbreaking work revealed a new and never before seen pathway for lactate metabolism, showing that there are multiple pathways for lactate removal after exercise (Brooks & Gaesser, 1980). The passive sink phenomenon is one such revolutionary pathway. The importance of these pathways is still a point of debate among physiologists (Kelley, Hamann, Navarre, & Gladden, 2002). The role of resting musculature as an endpoint for lactate metabolism is a novel concept that is gaining recognition.

The focus of this study is to further confirm the presence of the passive sink phenomenon in the lower extremity, which was first visualized by Comeau (2011) in the upper extremity, by applying similar experimental procedures. In so doing, this study will have two-fold benefits: first, the base of knowledge regarding lactate utilization in resting muscle will be broadened, which will benefit the field of physiology and spark more research into this interesting and unique phenomenon. Second, this study will provide evidence for clinicians to correctly choose a sample site close to the exercising muscle, which should systematically eradicate misleading results and lead to more
accurate training prescriptions, resulting in improved athletic performance (Comeau, et al., 2011).

**DELIMITATIONS**

This study will be delimited to:

1. Seven healthy, male subjects who are currently participating in a non-specific mixture of both anaerobic and aerobic conditioning.
2. Subjects who did not consume caffeine or any other stimulating substances 12 hours prior to the testing bout.
3. Subjects who were advised to refrain from exercise in the 24 hours prior to the testing bout.
4. Each subject was briefed on experimental procedure and signed an informed consent document prior to testing.
5. All testing was performed using the same SciFit (SCIFIT, Tulsa, OK) upper body ergometer at the Marshall University Recreation Center in a private laboratory setting.
6. Anthropomorphic measurements were determined by height and weight measurements. Height and weight were measured to the nearest inch and kg, respectively.
7. Blood samples were drawn from the finger and toe, using spring loaded Fisher Brand Unistik2, extra single use capillary sampling devices (Fisher Healthcare, Houston, TX). All blood samples were drawn into heparinized capillary tubes (Fisher Scientific, Pittsburgh, PA), and transferred into vials from the YSI 2315 Blood Lactate Preservative Kit (Yellow Springs, OH).
The blood lactate concentration was then analyzed using the YSI 2300 STAT plus-lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH).

LIMITATIONS

This study will be limited by the following:

1. The examiners cannot thoroughly control the subjects’ compliance to the pre-exercise restrictions set forth in the delimitations.

2. The subjects’ compliance to the pre-exercise restriction on caffeine and other stimulants cannot be thoroughly controlled.

3. The collection of a resting sample of blood lactate could cause the subjects discomfort, which could lead to apprehension of the following experimental procedure. This, in turn, could decrease their exercise performance.

4. The reporting of pre-testing conditioning level and training volume is subjective and could therefore vary among participants in the study.

5. Though the legs were the inactive muscle investigated during this study, there is the possibility that the non-exercising leg was not completely inactive (Ahlborg, et al., 1975). Every effort was made to ensure inactivity of the muscles through subject positioning, but bracing was not used (Smith, Doherty, Drake, & Price, 2004).

ASSUMPTIONS

It is assumed that all subjects who are volunteering for this study completed the health questionnaire accurately and were in compliance with all pre-testing requests prohibiting caffeine and exercise. It is also assumed that all subjects did complete the testing protocol and gave their maximal effort. For the purposes of this study, it is
assumed that during arm cycle ergometry, the upper body is the active component of the body while the lower body is the inactive component. Finally, it is assumed that all subjects were adequately acclimated to the testing procedures and that any outside stressors or apprehension did not negatively affect their performance during testing.

**HYPOTHESIS**

\( H_0 \): There will not be a significant difference in the blood [La\(^-\)] between the two sample sites after arm cycling to volitional exhaustion.

\( H_1 \): There will be a significant difference in the blood [La\(^-\)] between the two sample sites after arm cycling to volitional exhaustion.

**DEFINITION OF TERMS**

*Lactate Threshold* - The exercise intensity at which blood [La\(^-\)] begins to increase abruptly and progressively, or the exercise intensity where some fixed blood [La\(^-\)] is achieved (Gladden, 2004)

*Adenosine triphosphate (ATP)* - A molecule which is considered to be the energy currency of living organisms (McArdle, Katch, & Katch, 2010)

*Onset of blood lactate accumulation (OBLA)* - An increase in blood lactate equal to 4.0 mmol (McArdle, et al., 2010)
Chapter 2

REVIEW OF LITERATURE

Lactate, as it is commonly known, is actually lactic acid, which dissociates into a lactate anion (\(\text{La}^-\)) and a hydrogen ion (\(\text{H}^+\)) under normal physiological pH levels (Gladden, 2004). This substrate is utilized during exercise, and has been studied extensively (Ahlborg, et al., 1975; Astrand, Hultman, Juhlin-Dannfelt, & Reynolds, 1986; Brooks, 1986; Brooks & Gaesser, 1980). Aside from being the subject of much investigation, lactate is also a subject of much debate. Over the years, lactate has been assumed to play many different roles during exercise. One great misconception about lactate is that it causes muscle fatigue and is a dead end metabolic waste product (Gladden, 2004). Other past roles assigned to lactate include providing the energy necessary for muscles to contract, and the causative agent of oxygen debt (Gladden, 2004). Though these misconceptions have been invalidated by years of experimentation and study, most athletes and lay persons still believe that lactate causes their soreness and muscular pain. These misconceptions will be further addressed in this review of literature, as well as the studies that disproved them.

Lactate production results from muscle glycolysis and has traditionally been associated with anaerobic conditions during exercise (Brooks, 1986). However, lactate has also been shown to be produced under aerobic conditions (Brooks, 1986). There is a correlation between exercise intensity (metabolic rate) and \([\text{La}^-]\) (Brooks, 1986). Like most physiological processes, lactate appearance and disappearance usually occurs in a balanced fashion. However, when exercise intensity increases to a certain point, the energy demand placed upon the body cannot be met by the available energy, which leads
to a large accumulation of lactate in the blood and musculature (Astrand, et al., 1986; Brooks, 1986)

Theories of Lactate Accumulation

There have been many theories proposed to explain the physiological accumulation of blood lactate levels. The lactate threshold, the point in which blood lactate levels become elevated beyond lactate removal capabilities during incremental exercise is generally the point around which most of these hypotheses are centered (Gladden, 1996). First, there is the “traditional” hypothesis, which is known as the anaerobic threshold or muscle hypoxia hypothesis (Wasserman, 1984, 1986; Wasserman, Whipp, Koyl, & Beaver, 1973). This hypothesis essentially states that with an increase in exercise intensity, there is a concurrent increase in motor unit recruitment and oxygen demand is outpaced by oxygen supply (Gladden, 1996). Adenosine triphosphate (ATP) must be produced more quickly therefore; the tissues switch from oxidation to anaerobic glycolysis in order to match supply and demand for oxygen (Gladden, 1996).

Other more recent theories have become more widely accepted in response to evidence that lactate accumulation isn’t caused singularly by oxygen limitation in the mitochondria. One such theory is known as the “multiple factor” theory. This theory hypothesizes that the activity of the sympathoadrenal system, biochemical regulatory processes, and added recruitment of type FR (fast, fatigue resistant) and FF (fast, fatigable) motor units all interact to stimulate the production and inhibit the removal of blood lactate (Gladden, 1996). This theory maintains that the mitochondria can function optimally with muscle oxygen levels occurring during exercise, sufficient to increase \([\text{La}^-]\), which is in direct conflict with the traditional (hypoxic) theory (Gladden, 1996).
Finally there is the “unifying” theory, known as near-equilibrium steady state, which proposes to reconcile the “traditional” and “multiple factor” theories, by considering oxidative phosphorylation and oxygen levels as rate limiting steps for ATP synthesis, although not excluding the factors mentioned in the multiple factor theory (Gladden, 1996).

Although there are conflicting theories regarding how it occurs, the accumulation and resultant clearance of lactate in blood and muscle during exercise is a physiological certainty. The rate at which these occur is directly proportional to exercise intensity.

*Lactate Transportation*

During exercise, blood [La\(^{-}\)] increases progressively at the initial work load and as exercise intensity increases, the increase of blood [La\(^{-}\)] occurs more rapidly (Gladden, 2004). When the production of lactate exceeds the clearance of lactate, there is an increase in the [La\(^{-}\)] in both the muscle and blood (Gladden, 2004). The real question is what is the metabolic fate of this lactate after accumulation during exercise? The lactate shuttle hypothesis was proposed by Brooks (1986) to explain how lactate is formed and distributed. Essentially this theory posits that the shuttle operates within the intermediary metabolic processes of various tissues, which can occur in all physiological conditions (Brooks, 1986; Gladden, 2004). This hypothesis has dispelled the incorrect, but long held notion that lactate is nothing more than a dead end metabolic waste product that causes muscular fatigue under hypoxic conditions (Gladden, 2004). By approaching lactate as an intermediary metabolite that is diffusible, and can be rapidly exchanged among tissue compartments, the role of skeletal muscle in lactate metabolism has taken center stage (Gladden, 2004). Although skeletal muscle was previously thought to be
simply a producer of lactate, it is now seen as both a major component in the lactate shuttle, as well as a consumer, utilizing lactate (Gladden, 2004). Poortmans et al., (1978) suggests that resting skeletal muscle plays an integral part in the removal of blood lactate during intense bouts of exercise, which, when combined with the previously suggested theories, gives credence to the role of skeletal muscle as a passive sink. However, the lactate is also distributed to the liver, cardiac and active skeletal muscles for oxidation to provide substrates during the exercise bout (Brooks, 1986).

A key component of this “intracellular” lactate shuttle is the ability to provide lactate as a “mobile fuel” during steady state exercise (Gladden, 2007). This intracellular theory, as proposed by Brooks (1986), could revolutionize our understanding of the metabolic fate of lactate, and if it can be proven would cause our current biochemical understanding to be completely revised (Gladden, 2007). Gladden (2007) concisely summarizes the idea behind this intracellular shuttle:

“The cytosolic activity of the enzyme lactate dehydrogenase (LDH) is so high that pyruvate to La\(^{-}\) conversion is prevalent, making La\(^{-}\) the primary end product of glycolysis even under aerobic conditions. Lactate would then diffuse to mitochondria and into the mitochondrial matrix via facilitated diffusion across the inner membrane with the assistance of a monocarboxylate transporter (MCT). In the matrix, lactate would be converted back to pyruvate in a reaction catalysed by intramitochondrial LDH” (Gladden, 2007). This reaction, if it could be quantified, would in part explain and support the passive sink.
The Passive Sink

During exercise, the concentration of lactate is due to the balance of lactate manufacture and lactate disposal, which is a dynamically occurring process (Poortmans, et al., 1978). As previously mentioned, cardiac muscle, active muscle and the liver are all sites within the body where lactate can be eliminated during and after intense bouts of exercise (Brooks, 1986; Brooks & Gaesser, 1980; Kelley, et al., 2002). Non-exercising skeletal muscle’s role in lactate uptake has been suggested to be that of a “passive sink” by Kelley (Kelley, et al., 2002), who showed that resting skeletal muscle uptakes lactate in conditions of elevated arterial [La⁻], and stores it passively, without much lactate metabolism.

Skeletal muscle that is active is known to take up lactate from blood during exercise; however, it has been shown that lactate can also end up in association with blood flow to the inactive muscle, in which only a small percentage of the lactate is metabolized, while the rest is turned back into the circulation during exercise, and throughout the recovery process (Poortmans, et al., 1978).

The transport of lactate from the cytosol where it is produced, to the mitochondria where it is consumed, in the intracellular lactate shuttle means that the mitochondrial area is serving as a sink, into which there is a net lactate uptake from the blood into the resting musculature (Gladden, 1996). Due to the large mass of skeletal muscle, as well as its metabolic capacity, it is clear that muscle is a large functional component of the lactate shuttle (Poortmans, et al., 1978). Exercise training leads to an improved ability to utilize lactate, which could lead to faster or improved ability to transport it across the sarcolemmal membrane and into blood, where it could be oxidized by muscle or transported to sites
distant to the active muscle (Gladden, 1996). In their study on canine skeletal muscle, Kelley et al. (2002) defined the passive nature of the non-exercising muscle, stating that lactate is not oxidized in the non-exercising muscle, nor is it stored as fuel there. The non-exercising muscle does not serve as a sink for very long after the exercise bout, and the \([\text{La}^-]\) declines rapidly until about 15 minutes after exercise stoppage (Catcheside & Scroop, 1993; Poortmans, et al., 1978).

Comeau and colleagues (2011) were able to visualize this phenomenon using two sample sites and a cycling protocol to volitional fatigue. Their study was the first of its kind, utilizing two sampling sites (a finger and toe) associated with an exercising and non-exercising muscle group, and overlaying the values for comparison. The findings of this unique study were significant, showing differences between the two sites at several time points (Comeau, et al., 2011). However, the study by Comeau and colleagues (2011) did not perform the reverse of their experiment, and perform an upper extremity exercise bout with samples drawn from the non-exercising lower extremity, though they acknowledge that it is reasonable to conclude from their data that the passive sink phenomenon would be visualized with this reverse procedure. It is the intriguing possibility of visualizing the passive sink in the non-exercising lower extremity during upper extremity exercise presented by Comeau’s initial study, as well as the evidence of its existence in other literature (Brooks, 1986; Comeau, et al., 2011; Poortmans, et al., 1978) that shaped the central research question of this study. The practical applications of using this phenomenon to more accurately choose sample sites for physiological lactate assessments, taking into consideration their proximity near or away from the
exercising muscle would result in more accurate training protocols, performance
evaluations and improved athletic performance.

_Lactate Testing_

Blood lactate analysis has long been utilized as a method for assessment of an
individual’s training status, as well as for the prescription of a training protocol to
improve that individual’s athletic performance (el-Sayed, George, Wilkinson, Mullan,
Fenoglio, & Flannigan, 1993; Garland & Atkinson, 2008; Moran, et al., 2012). One of
the many benefits of lactate testing is the ease with which the samples can be collected
via capillary micro-punctures. Capillary blood samples are the method of choice for
blood lactate sampling because the lactate levels in capillary blood closely mirror values
in arterial blood, and are less invasive to acquire (Feliu, Ventura, Segura, Rodas, Riera,
Estruch, Zamora, & Capdevila, 1999). Capillary samples have been found to be more
easily tolerable for the subjects and more readily obtainable for the investigator during
exercise conditions, and require less advanced technical skill to perform (el-Sayed, et al.,

The reason we test blood lactate is that it is an indirect measure of the energy
production within the muscles, which allows us to make performance assumptions based
on lactate analysis results. Energy within the human body can be generated through
metabolic pathways that operate with oxygen (aerobic) or without oxygen (anaerobic).
There is a point, known as the onset of blood lactate accumulation (or OBLA) at which
the level of lactate in the blood has increased to 4 mmol·L⁻¹ which indicates that lactate
production and clearance are not at equilibrium (el-Sayed, et al., 1993). During exercise
at heavy intensity, [La⁻] can rise to 20-30 mmol·kg⁻¹ of wet muscle weight marking a shift
from oxidative energy systems to anaerobic pathways (Karlsson, et al., 1975). Because the level of lactate in blood and muscle will increase with acidosis due to the dissociation of a hydrogen ion, use of the OBLA as an indicator for performance is an accepted practice (el-Sayed, et al., 1993; Karlsson, et al., 1975). In the field of exercise physiology, a common method for using blood lactate results to manipulate training is to train the athlete at or above the OBLA point (Spurway, 1992).

As previously mentioned, both aerobic and anaerobic systems can be tested and trained using blood lactate values. Lactate is the product that results from a breakdown of glucose, and and glycogen, two sugars that exist in the human body, and this reaction occurs anaerobically within the first 2-3 minutes of exercise (Brooks, Fahey, White & Baldwin, 2000). Anaerobic conditions result in higher levels of lactate accumulation in the blood, as this system is rapidly recruited for energy (Gladden, 2004). However, quantifying an individual’s anaerobic capacity can be difficult.

Aerobic (endurance) type exercise is very well measured by blood lactate levels. The aerobic system becomes the predominant energy system during exercise bouts lasting longer than 2-3 minutes, and requiring effort of low to moderate exercise intensity. Therefore, the determination of a protocol to accurately assess the fitness of the aerobic system has been investigated. Comparison of incremental and steady state exercises has shown that incremental stages of increasing intensity lead to a blood \([\text{La}^-]\) sufficient to assess the development of the aerobic energy system (Garland & Atkinson, 2008; Smith, et al., 2004). Also, it is important to keep in mind that protocol is not the only variable that influences blood \([\text{La}^-]\), but also exercise duration and work rate play a role (Garland & Atkinson, 2008).
The use of a Borg Rating of Perceived Exertion (RPE) scale has been widely used to measure exercise intensity, and there is a correlation between the absolute RPE values reported, the lactate threshold, and blood [La\(^{-}\)] (Boutcher, Seip, Hetzler, Pierce, Snead, & Weltman, 1989; Chen, Fan, & Moe, 2002). Therefore, this scale will be used to monitor and estimate lactate threshold and blood [La\(^{-}\)] during the course of this study.

The muscle group that is undergoing the exercise is another factor that influences the physiological responses attained from incremental testing. The muscle mass of the arm has been shown to have higher levels of blood lactate when exercising to volitional exhaustion, which may be due to the fact that the muscle mass of the arm is smaller than that of the leg, and requires a gripping component that is not present in leg cycle ergometry (Smith, et al., 2004), implying that the mass of the active tissue, can increase the blood [La\(^{-}\)], resulting in increased muscular [La\(^{-}\)] (Baker, et al., 2002). This metabolic response of the working muscle is of interest to physiologists, however, the interest of this study is in the difference in blood [La\(^{-}\)] in the metabolic response between active and inactive muscle. Arm exercise has been found to cause a decrease in the vascular resistance and an increase in blood flow, thus there is increased oxygen uptake in the non-exercising leg during upper extremity exercise (Ahlborg, et al., 1975). There is considerable net uptake of lactate and other bloodborne substrates via diffusion into the non-exercising muscles, which has implications for the metabolism of lactate (Ahlborg, et al., 1975).

An understanding of the metabolic and physiological processes associated with arm cycle ergometry is important because there is a wide range of clinically relevant applications for this information. Arm cycle ergometry is an exercise modality that is
specifically beneficial to those individuals who participate in arm dominant sports such as rowing, kyaking, “grinding”, as well as wheel chair sports and activities of daily living for those who are paraplegic and do not have the use of their legs. (Price, Bottoms, Smith, & Nicholettos, 2011). Arm cycle ergometry is also a valuable clinical tool for use with individuals who suffer from coronary heart disease, or other physiological or orthopedic limitations to lower extremity exercise such as peripheral vascular complications, ischemia or intermittent claudication (Forsyth & Farrally, 2000; Garland & Atkinson, 2008; Smith, et al., 2004; Wecht, Marsico, Weir, Spungen, Bauman, & De Meersman, 2006).

In order to visualize differences in blood [La−], the importance of sample site selection cannot be overemphasized. It has been previously stated that there are multiple widely utilized sites for common sampling procedures. Differences between lactate values from various sampling sites have implications on training and athletic performance by influencing OBLA and exercise intensity (Forsyth & Farrally, 2000). In the most recent work to compare blood lactate from two sample sites, Moran and colleagues (2012) found no significant difference between blood [La−] taken from the finger and ear, but rather a strong positive relationship leading them to the conclusion that the finger and ear can be used interchangeably as sites for lactate measurement in the upper extremity. These results directly conflict with the outcome of Feliu’s experimentation, which found a significant difference between blood [La−] levels found in the fingertip and ear (Feliu, et al., 1999). The ear has been found to have lower levels of blood [La−] than the finger by several investigators (Dassonville, Beillot, Lessard, Jan, Andre, Le Pourcelet, Rochcongar, & Carre, 1998; Feliu, et al., 1999). Due to the
inequalities of \([La^-]\) values between the finger and ear sites, the earlobe is excluded as a sample site for this study. Garland (2008) has also proved that the toe is a well tolerated sample site and is fairly readily accessible for data collection, especially in activities such as rowing, or in this case arm cycle ergometry.

The role of the non-exercising muscle on lactate metabolism is once again the confounding factor to the establishment of standard protocols for sample site selection. In a study that compared treadmill and arm crank ergometry with lactate levels being collected from the toe and finger, higher lactate levels were reported in the toe samples during the arm crank ergometry exercise. This study provides compelling evidence of both the passive sink phenomenon as well as confirms that lactate values are variable not only among sample sites, but, depending on the mode of exercise, (ergo, the active muscle group) (Dassonville, et al., 1998).

Finally, the relationship between exercise intensity and blood \([La^-]\) is evident in the study by el-Sayed (1993), which shows that with increasing exercise intensity, the difference in blood \([La^-]\) increases between sample sites. Thus, the exhaustive protocol utilized in this study should be of sufficient intensity to elicit a highly visible difference in blood \([La^-]\) between sample sites, allowing us to clearly visualize the passive sink phenomenon.

**Summary**

The previous research was presented in order to highlight the evolution of our understanding of blood lactate. The resources used to compile this literature review were chosen to provide multiple perspectives on the scientific, historical, experimental and practical aspects related to blood lactate testing and analysis. This comprehensive
approach was intended to address aspects relevant to this study.
Chapter 3

METHODS

SUBJECT RECRUITMENT

Subjects for this study were 7 healthy, non-specifically trained college aged males, who participated on a voluntary basis. Each subject had exercise history and used a combination of aerobic conditioning and anaerobic training on a regular basis (2-3 bouts/week). All subjects were pre-screened and risk stratified according to the standards of the American College of Sports Medicine (ACSM). Subjects were only accepted for this study if they meet the ACSM low risk criteria. Subjects were excluded from the study if they presented with known peripheral neuropathy or peripheral vascular disease of the lower extremity, due to an increased risk of complications during this study. All subjects were oriented to the procedures and risks of this study prior to any exercise testing. This study received IRB approval (Appendix A).

INFORMED CONSENT

All subjects completed an informed consent (Appendix B). This document gave detailed information on the testing procedures, time requirements, potential risks and benefits of participation in this study, confidentiality precautions and the right to withdraw from testing at any time. All subjects must understand and sign the informed consent prior to undergoing any exercise testing. The informed consent states that there will be no compensation for any injury sustained over the course of this study.

INSTRUMENTATION

Blood samples were drawn from the finger and toe using spring loaded Fisher Brand Unistik2, extra single use capillary sampling devices (Fisher Healthcare, Houston,
All blood samples were drawn into Fisher Brand heparinized capillary tubes (Fisher Scientific, Pittsburgh, PA), and transferred into vials from the YSI 2315 Blood Lactate Preservative Kit (Yellow Springs, OH). The blood lactate levels were then analyzed using the YSI 2300 STAT plus-lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Testing was performed using a SciFit Pro 1000 arm cycle ergometer (SCIFIT, Tulsa, OK). Ratings of Perceived Exertion (RPE) were obtained using a standard Borg scale, which ranges from 6 to 20. Anthropometric measurements were taken using electronic scales (Seca Alpha, Model 770). These measurements will be taken for purely demographical purposes.

**PROCEDURE**

Subjects were instructed to refrain from eating at least 2 hours prior to exercise, from consuming any caffeine products for 12 hours prior to exercise and to abstain from exercise for 24 hours prior to participating in the study. Subjects reported to the Marshall University Recreational Center’s laboratory, where they were oriented to the testing equipment and procedure. Prior to testing, the subject’s anthropometric measurements will be taken, to the nearest 0.1 cm and 0.1 kg, using a measuring tape and an electronic scale. The subjects were then instructed to rest in a supine position on a padded table for 30 minutes. After the resting period, baseline blood samples were drawn from the distal portion of the subject’s third or fourth phalanx of the non-dominant hand and from the great toe on the ipsilateral side. After bleeding at the sample sites was stopped with gauze pads, the subject was be allowed to be seated in front of the Sci Fit Pro 1000 cycle ergometer.
Subject positioning followed procedures utilized by Smith et al, seating the subject so that the crank shaft of the ergometer was level with the subject’s shoulder joint. Subject was also instructed to sit back firmly in the seat to maintain proper biomechanics throughout the testing, ensuring that the subjects’ elbows would be slightly bent when the arm is at the endpoint of the cranking motion. The legs were not strapped down, but subjects were instructed to keep their feet flat on the foot rests, and placed in front of them throughout the test in order to ensure resting of the extremity (Smith, et al., 2004). Subjects then began testing with a 1 minute warm up period to familiarize themselves with the functions of the ergometer. This warm up was performed against 10 W of resistance, at a self-selected cadence. Arm ergometry testing then commenced following a 15-W·min⁻¹ incremental exercise protocol at 60 rpm to volitional fatigue or failure to maintain the 60 rpm for 10 seconds (Wecht, et al., 2006). A Borg Scale for rating perceived exertion was also utilized to monitor the subjects throughout the test, due to the fact that RPE values have an association with lactate threshold and have been shown to be effective means for regulating exercise intensity (Boutcher, et al., 1989; Chen, et al., 2002).

Blood was collected using the previously mentioned method and locations immediately post exercise and at 5 minute intervals for 30 minutes post exercise. Blood samples were drawn at approximately the same time, in order for the lactate accumulation data to be as accurate as possible for that time point.

**BLOOD SAMPLING AND ANALYSIS**

All blood sampling was conducted on the phalanges of the non-dominant hand and from the great toe of the ipsilateral foot. All sample sites were cleaned using
antiseptic 70% isopropyl alcohol prep swabs prior to puncture. Pressure was then applied proximally to the sample site to pool the blood, and the site was pierced with a spring loaded lancet. The initial blood was wiped away with a 70% isopropyl alcohol swab to avoid contaminating the sample. The site then yielded two full 50 µL heparinized capillary tubes. Blood samples were collected as quickly as possible to eliminate unwanted lactate accumulation within the sample. The blood was then transferred to time labeled tubes containing blood lactate preservatives and an anti-coagulant (YSI 2315 Blood Lactate Preservative Kit, YSI, Yellow Springs, OH). The blood was then analyzed for total blood lactate concentration using the YSI 2300 STAT plus- lactate analyzer (Yellow Springs, OH).

**STATISTICAL ANALYSIS**

The data were compiled using Microsoft Excel (2010) and analyzed using SPSS (version 19.0). A two-way, 2 X 7 (site X sample time) within-subjects repeated-measures ANOVA was used to determine significance differences between the \([La^-]\) obtained from the two sample sites in five minute intervals during the cool-down ride. Post hoc pairwise differences, calculated by subtracting the fingertip blood lactate value from the great toe lactate value for each site, were determined for each time variable. Paired samples t-tests using the calculated pairwise differences for each time variable were conducted for every possible time combination to determine where the differences occurred. Significance was established at an alpha level of \(p \leq 0.05\).
Chapter 4

RESULTS

A total of 7 subjects were tested throughout the course of this study. The subjects were all college aged males whose demographics (mean ± SD) are included in Table 1. A two-way 2 x 7 (site x sample time) within- subjects repeated-measures ANOVA determined no significant interaction effect. A significant time main effect did exist with Wilks’ Lambda = .032 (F<sub>6,7</sub> = 35.114, p=0.000). There was a cross over effect during the post-exercise period which began with elevated blood [La] in the finger immediately post exercise, with toe blood lactate [La] surpassing the finger at the 15 minute time point. This overall cross over effect is visible in Figure 1. Trend lines were calculated for each data set in an attempt to extrapolate small changes in [La]. The trend line for the toe mean was y = 0.206x + 6.1695. The trend line for the finger mean was y = 0.6425x + 8.0826. These trend line values are also visible on Figure 1. These small changes represent what is believed to be a hidden passive sink for lactate due to the large muscle mass involved in the inactive muscle versus the smaller muscle mass in the active muscle.
Figure 1. Finger and toe [La\(^-\)] during recovery post-exercise (mean ± SD).
Table 1. Descriptive statistics of subject (mean ± SD).

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Chapter 5
DISCUSSION

The role of inactive skeletal muscle serving as a “passive sink” for blood lactate released by an exercising muscle group has been proposed by several authors (Ahlborg, et al., 1975; Brooks, 1986; Buckley, Scroop, & Catcheside, 1993; Catcheside & Scroop, 1993; Comeau, et al., 2011; Dassonville, et al., 1998; Poortmans, et al., 1978). The findings of Comeau et al. (2011) further enforced the presence of this phenomenon, exhibiting higher [La−] values in the finger samples obtained after a bout of lower extremity exercise than [La−] values taken from the toe at the same time.

This study was designed to mimic the previously published work by Comeau et al. (2011), in an attempt to confirm the occurrence of the passive sink in reverse, utilizing upper extremity exercise with the lower extremity as the resting component. The findings of this study revealed no significant differences between sites at any time point. There are several factors that could have contributed to this outcome.

Of interest is the size of the working muscle mass. Skeletal muscle is known to have a large role in lactate production and utilization due to its large mass and capacity to metabolize carbohydrate substrates, particularly lactate (Gladden, 2004). Therefore, it would be reasonable to assume that the size of the muscle mass being utilized for exercise would play a role in the extent of lactate change in the resting musculature (Karlsson, et al., 1975). As the upper extremity calls upon a markedly smaller active muscle mass than that of the lower extremity, the biochemical changes evidenced in the non-working muscle would be of a smaller magnitude than the changes seen in previously published data (Comeau, et al., 2011; Karlsson, et al., 1975; Smith, et al., 2004). The slope of the finger mean line seen in our study (see Figure 1) is very closely
related to the slope of the finger mean line seen in previously published data (Comeau, et al., 2011). This is interesting because in our study the arm was the active muscle group and in the previously published study, it was serving as a “passive sink”. We would not expect the slope of our arm value to be this steep, due to the active nature of the arm musculature. Also, in Figure 1, the relationship of the immediate post toe value is greatly elevated when compared with previously published data, which is evidence of a shift of lactate toward the non-exercising lower extremity (Comeau, et al., 2011). We assume that this shift is due to pooling of the blood \[\text{[La]}^\cdot\text{]}\]. This leads us to believe that the “passive sink” does occur in the lower extremity during upper extremity exercise, though it is not as pronounced as the sink evidenced in the upper extremity by Comeau et al. (2011).

Another consideration as to why we did not see a pronounced interaction effect between sites could be related to the selection of protocol. The cranking component of upper body ergometry has significant impact on physiological responses (Price, et al., 2011). Our chosen imposed cadence of 60 rpm is considered to be a slow crank rate, which has been found to be appropriate for exercise at low intensity (Price, et al., 2011). However, as the intensity of our ramp protocol increased, this slow crank rate could have contributed to local fatigue of the subjects’ upper extremity (Price, et al., 2011). Also, keeping the crank rate constant at 60 rpm resulted in greater resistance per given power output towards the end of the exercise bout (Price, et al., 2011). The \[15\cdot\text{W}\cdot\text{min}^{-1}\] protocol utilized by this study was primarily dictated by the ability of the SCIFIT Pro 1000 to be controlled in 15 W increments. This resistance resulted in our subjects reaching volitional fatigue in less than 10 minutes. Because the biochemical response to exercise
is dependent upon both work rate and duration of work, the blood [La\(^{-}\)] response seen in this study may not be reflective of the muscles true work in our chosen protocol (Garland & Atkinson, 2008). The combination of chosen crank rate and work load could have led to exhaustion before compartmental fluid shifts were able to occur. Therefore, it is possible that the lactate responses occurring in our study were sub-maximal in nature.

Though the results of this study were not significant, they were provocative, and show a definite trend towards significance. The passive sink phenomenon may occur in a smaller magnitude in the lower extremity during upper extremity exercise due to the smaller muscle mass of the upper extremity. The findings of this study demonstrate that there is still much to be understood about the metabolic fate of lactate during and after maximal exercise. In addition to sample site considerations when utilizing blood [La\(^{-}\)] for performance evaluation and prescription, this study shows that different modes of exercise should be taken into consideration when utilizing blood [La\(^{-}\)] values. Local fatigue of musculature due to selection of an overly vigorous protocol could result in [La\(^{-}\)] values that are not truly representative of the muscle’s work (Comeau, et al., 2011; Garland & Atkinson, 2008; Price, et al., 2011; Smith, et al., 2004).

**PRACTICAL APPLICATION**

When utilizing lactate as an indicator of athletic performance, samples should be taken from a site that is in closer proximity to the active musculature, which will allow for the most accurate assessment of blood [La\(^{-}\)], leading to better training prescriptions and outcomes.
Bibliography


Appendix A

IRB
March 26, 2012

Matthew Comeau, PhD
Marshall University, School of Kinesiology

RE: IRBNet ID# 317498-1
At: Marshall University Institutional Review Board #1 (Medical)

Dear Dr. Comeau:

Protocol Title: [317498-1] VISUALIZATION OF THE PASSIVE SINK PHENOMENON IN NON-EXERCISING LOWER EXTREMITY MUSCLE USING TWO SAMPLING SITES: CONSEQUENCES FOR ASSESSMENT AND TRAINING

Expiration Date: March 26, 2013
Site Location: MU
Submission Type: New Project APPROVED
Review Type: Expedited Review

In accordance with 45CFR46.110(a)(2)&(4), the above study and informed consent were granted Expedited approval today by the Marshall University Institutional Review Board #1 (Medical) Chair for the period of 12 months. The approval will expire March 26, 2013. A continuing review request for this study must be submitted no later than 30 days prior to the expiration date.

If you have any questions, please contact the Marshall University Institutional Review Board #1 (Medical) Coordinator Trula Stanley, MA, CIC at (304) 696-7320 or stanley@marshall.edu. Please include your study title and reference number in all correspondence with this office.
Appendix B

Consent Form
Informed Consent to Participate in a Research Study

Visualization of the Passive Sink Phenomenon in Non-Exercising Lower Extremity Muscle Using Two Sampling Sites: Consequences for Assessment and Training

Matthew J Comeau, PhD, ATC, CSCS, Principal Investigator

Introduction

You are invited to be in a research study. Research studies are designed to gain scientific knowledge that may help other people in the future. You may or may not receive any benefit from being part of the study. There may also be risks associated with being part of research studies. If there are any risks involved in this study then they will be described in this consent. Your participation is voluntary. Please take your time to make your decision, and ask your research doctor or research staff to explain any words or information that you do not understand.

Why Is This Study Being Done?

The purpose of this study is to determine the effects of a 30 second cycle sprint performance on blood lactate concentrations.

How Many People Will Take Part In The Study?

About 15 people will take part in this study. A total of 20 subjects are the most that would be able to enter the study.

What Is Involved In This Research Study?

Before you begin the study, the following would occur:

- Report to the Human Performance Laboratory in Gullickson Hall
- Read consent form and sign if willing to participate
- Height, weight, and age will be recorded.

During the study, you will:

- Lie supine and rest for 30 minutes
- A very small sample of blood will be obtained from the ring finger and great toe of the non-dominant side
- An aerobic test using an upper body ergometer to exhaustion will be performed.
- You will remain seated for 30 minutes after the exercise bout and blood samples will be obtained every 5 minutes

How Long Will You Be In The Study?

You will be in the study for about approximately 1.5 to 2 hours.

Subject’s Initials _____
You can decide to stop participating at any time. If you decide to stop participating in the study, we encourage you to talk to the investigators or study staff to discuss what follow up care and testing could be most helpful for you.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest; if you do not follow the study rules; or if the study is stopped.

**What Are The Risks Of The Study?**

Being in this study involves some risk to you. You should discuss the risk of being in this study with the study staff.

You should talk to your study doctor about any side effects that you have while taking part in the study.

Risks and side effects related to the sampling of blood: pricking sensation in the ear, finger and toe.

Risks and side effects related to the sprint performance: possible light headedness and nausea

There will be no compensation if any injuries occur during the completion of this study.

**Are There Benefits To Taking Part In The Study?**

If you agree to take part in this study, there may or may not be direct benefit to you. We hope the information learned from this study will benefit other people in the future. The benefits of participating in this study may be: to determine the effects of a 30 second cycle sprint performance on blood lactate concentrations.

**What About Confidentiality?**

We will do our best to make sure that your personal information is kept confidential. However, we cannot guarantee absolute confidentiality. Federal law states that we must keep your study records private. Nevertheless, certain people other than your researchers may also need to see your study records. By law, anyone who looks at your records must keep them completely confidential.

Those who may need to see your records are:

- Certain university and government people who need to know more about the study. For example, individuals who provide oversight on this study may need to look at your records. These include the Marshall University Institutional Review Board (IRB) and the Office of Research Integrity (ORI). Other individuals who may look at your records include: the federal Office of Human Research Protection. This is done to make sure that we are doing the study in the right way. They also need to make sure that we are protecting your rights and your safety.

If we publish the information we learn from this study, you will not be identified by name or in any other way.

**What Are The Costs Of Taking Part In This Study?**

Subject’s Initials ________
There are no costs to you for taking part in this study. All the study costs, including any study medications and procedures related directly to the study, will be paid for by the study. Costs for your regular medical care, which are not related to this study, will be your own responsibility.

**Will You Be Paid For Participating?**

You will receive no payment or other compensation for taking part in this study.

**What Are Your Rights As A Research Study Participant?**

Taking part in this study is voluntary. You may choose not to take part or you may leave the study at any time. Refusing to participate or leaving the study will not result in any penalty or loss of benefits to which you are entitled. If you decide to stop participating in the study we encourage you to talk to the investigators or study staff first to learn about any potential health or safety consequences.

**Whom Do You Call If You Have Questions Or Problems?**

For questions about the study or in the event of a research-related injury, contact the study investigator, Matthew Comeau, PhD at 696-2925 8:00 am – 5:00 pm. You should also call the investigator if you have a concern or complaint about the research.

For questions about your rights as a research participant, contact the Marshall University IRB#1 Chairman Dr. Henry Driscoll or ORI at (304) 696-7320. You may also call this number if:
- You have concerns or complaints about the research.
- The research staff cannot be reached.
- You want to talk to someone other than the research staff.

You will be given a signed and dated copy of this consent form.

**SIGNATURES**

You agree to take part in this study and confirm that you are 18 years of age or older. You have had a chance to ask questions about being in this study and have had those questions answered. By signing this consent form you are not giving up any legal rights to which you are entitled.

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Subject Name (Printed)  

Subject Signature  

Date

Person Obtaining Consent  

Date

Principal Investigator  

Date

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Site: 1 = finger, 2 = toe